STANDARD OPERATING PROCEDURE

FIELD COLLECTION OF ORGANISMS FOR TISSUE SPECIMENS FOR ANALYSIS OF TRACE METALS AND SYNTHETIC ORGANIC COMPOUNDS

(MARINE MUSSELS, FRESHWATER CLAMS, MARINE CRABS, AND MARINE/FRESHWATER FISH)

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SAMPLE COLLECTION AND PREPARATION (DFG Method 102)

1.0 SCOPE AND APPLICATION

1.1 The following procedures describe techniques for field collection and minor field processing of biological tissue samples used for the analysis of trace metals (TM) and synthetic organic compounds (SO), primarily in marine mussels, freshwater clams, and marine/freshwater fish and crabs.

2.0 SUMMARY OF METHODS

2.1 Collect mussels, clams, fish, or crabs. Mussels or clams to be transplanted are placed in polypropylene mesh bags and deployed. Mussels and clams to be analyzed for metals are double-bagged in plastic Ziploc™ bags. Each sample should be labeled with Date, Station Name, and any other information available to help identify the sample once in the lab. Bivalves to be analyzed for organics are wrapped in PE cleaned aluminum foil prior to placement in the Ziplocs™. Fish are wrapped whole or proportioned where necessary in cleaned Teflon sheets and subsequently placed into Ziplocs™. Crabs analyzed for metals and/or organics are double-bagged in plastic Ziploc™ bags.

2.1 After collection, samples are transported back to the laboratory in coolers with ice or dry ice. If ice is used, care must be taken to ensure that ice melt does not come into direct contact with samples.

2.2 Laboratory processing is carried out under “clean room” conditions, with a positive pressure filtered air supply, non-contaminating laboratory surfaces, and a supply of deionized and Type II water (MilliQ). Laboratory processing (dissection and homogenization) of tissue samples is described in detail in a separate SOP.

3.0 INTERFERENCES

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or elevated baselines, causing inaccurate analytical results. All materials should be demonstrated to be free from interferences under the conditions of the analysis by running method blanks initially and with each sample lot.

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4.0 APPARATUS AND MATERIALS

4.1 Polypropylene mesh, 76mm wide with 13mm mesh

4.2 Polypropylene mesh, 50mm wide with 7mm mesh

4.3 Nylon cable ties, 7/16” wide x 7” long

4.4 Inflatable Buoy, 30cm

4.5 Polypropylene line, 16mm

4.6 Screw in earth anchor, 4-6’ diameter

4.7 Anchor Chains

4.8 Stainless Steel Dive Knives

4.9 Ziploc™ Bags 9 ¾” x 14” x 4 mm

4.10 Heavy-duty plastic bags, 30 gal, 2 mm.

4.11 Micro detergent

4.12 Polyethylene gloves

4.13 Heavy duty aluminum foil

4.14 Permanent marking pen

4.15 Gummed waterproof labels

4.16 Disposable stainless steel blade scalpels (#10)

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4.17 Brinkmann Polytron model PT 10-35

4.18 Büchi Mixer B-400

4.19 Tall 500mL glass jars with Teflon lined lids

4.20 Polypropylene dissection jars, 125mL

4.21 Plastic ice chests

4.22 Plastic knives

4.23 Titanium rods

4.24 Shoe Covers

4.25 Lab coats

4.26 Daypacks

4.27 Gill nets (various sizes)

4.28 Trap nets (hoop or fyke nets)

4.29 Seines (various size mesh and lengths as appropriate)

4.30 Otter trawl (various widths as appropriate)

4.31 Backpack shocker (electro-fishing)

4.32 Boats (electro-fishing and/or for setting nets)

4.33 Dip nets

4.34 Cast nets (10’ and 12’)

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4.35 Polypropylene crab traps

4.36 Rods and reels

4.37 Other (minnow traps, set lines, throw nets, etc)

4.38 GPS

4.39 Depth finder

4.40 Data sheets

4.41 Dry ice or ice

4.42 Wading Gear

4.43 Stainless steel sediment grab sampler with epoxy coated weights.

4.44 Permanent marker

4.45 Scuba gear

4.46 camera

4.47 Teflon forceps

4.48 24 x 24 x 1/4 inch glass or Teflon sheet

4.49 Eight inch Teflon policemen, nine inch glass rods

4.50 Needle nose pliers

4.51 1000ml heavy duty beakers

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4.52 400ml heavy duty beakers

4.53 25ml beakers

4.54 Ear protection

4.55 Laboratory goggles

4.56 Tweezers

4.57 500ml Teflon squeeze bottles

4.58 250ml and 125ml Wheaton bottles with ground glass stoppers

4.59 Teflon sheets

4.60 Aluminum sheets

5.0 REAGENTS

5.1 ASTM Type II water (MilliQ)

5.2 Deionized Water (DI)

5.3 Petroleum ether (Baker)

5.4 Methanol (Baker)

5.5 6N Nitric Acid: to prepare, add 800mL concentrated HNO_3 (Baker) to 1200mL DI

5.6 6N Hydrochloric Acid: to prepare, add 1000mL concentrated HCl (Baker) to 1000mL DI

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

In the field, sources of contamination include sampling gear, dirty hands, grease from

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ship winches or cables, ship and truck engine exhaust, dust, and ice used for cooling. Efforts are made to minimize handling and to avoid sources of contamination. All sampling equipment will be made of non-contaminating materials and will be inspected prior to entering the field. Nets will be inspected for holes and repaired prior to being used. Boats (including the electroshocking boat) will be visually checked for safety equipment and damage prior to being taken into the field for sample collection. Samples are handled with polyethylene-gloved hands only. The samples are sealed in appropriate containers immediately. Mussels and clams to be analyzed for metals are double-bagged in plastic Ziploc™ bags. Bivalves to be analyzed for organics are wrapped in aluminum foil prior to placement in the Ziplocs™. Fish are wrapped in part or whole in Teflon and subsequently placed into Ziplocs™. Crabs analyzed for metals and/or organics are double-bagged in plastic Ziploc™ bags. Teflon sheet used to wrap fish is cleaned by soaking in micro™, acid and rinsed with petroleum ether. Aluminum foil used to wrap bivalves is cleaned prior to use by rinsing with petroleum ether or methanol or by heating in a kiln (500°c). Samples are frozen as soon as possible. In the field, dry ice is ideal, but regular ice may be used if it is sealed in watertight plastic bags.

6.1 A chain of custody form (Appendix A-1) will accompany all samples that are brought to the lab. All samples that are processed in the lab WILL receive a unique lab number for identification. This number is recorded in the Lab Number Log book, and is used to identify the sample while it is processed through the lab.

6.2 To avoid contamination, all equipment used in sample collection is thoroughly cleaned before each sample is processed. Ideally, instruments are made of a material that can be easily cleaned (e.g. Stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments are washed with a detergent solution, rinsed with ambient water, rinsed with MilliQ and finally rinsed with a high-purity solvent (methanol or petroleum ether). Solvent solutions are collected and taken back to the laboratory.

6.3 Tissue dissections in the field (used only for fish that are too large to be put whole into cleaned bags, as described further below) are carried out by or under the supervision of a qualified technical staff. Samples are handled with polyethylene gloves and changed between samples. Each organism is rinsed free of dirt with deionized water and handled with clean stainless steel, quartz, or Teflon instruments. The SO specimens only contact with pre-cleaned glass, aluminum foil or Teflon surfaces.

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7.0 PROCEDURE FOR FIELD COLLECTION OF ORGANISMS FOR TISSUE SPECIMENS

7.1 Sample collection - mussels and clams

7.1.1 The mussels to be transplanted (*Mytilus californianus*) are collected from Trinidad Head (Humboldt Bay Intensive Survey), Montana de Oro (Diablo Canyon Intensive Survey), and Bodega head (all other statewide transplants). The freshwater clam (*Corbicula fluminea*) source is Lake Isabella or the Sacramento River. Mussel and clam samples are analyzed for background contaminates prior to transplanting.

7.1.2 Polyethylene gloves are worn while prying mussels off rocks with stainless steel dive knives. Note: polyethylene gloves should always be worn when handling sample. Mussels of 55mm to 65mm in length are recommended. Fifty mussels are collected for each TM and each SO sample.

7.1.3 Collected mussels are carried out of collection site in cleaned nylon daypacks. For the collection of resident samples where only one or two samples are being collected the mussels are placed directly into a labeled Ziploc™ (TM) or cleaned aluminum foil (SO) and an additional Ziploc™.

7.1.4 Clams (*Corbicula fluminea*) measuring 20 to 30mm are collected by dragging the clam dredge along the bottom of the lake or river. The clams are poured out of the dredge into a 30 gallon plastic bag. 25-50 clams are needed for each TM and each SO sample.

7.2 Transplanted sample deployment

7.2.1 With polyethylene gloves, fifty transplant mussels are placed in each 76mm X 13mm polypropylene mesh bag. Each bag represents one TM or one SO sample. A knot is tied at each end of mesh bag and reinforced with a cable tie. On one end another cable tie is placed under the cable tie which will be used to secure the bag to the line for transplant deployment. The mussels in the mesh bag are divided into three groups of approximately equal size and sectioned with two more cable ties.

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7.2.2 Once bagged, the mussels are placed in a 30 gallon plastic bag and stored in a cooler (cooled with ice) for no more than 48 hours. The ice is double bagged in Ziploc™ bags to avoid contamination.

7.2.3 If samples are held for longer than 48 hours they are placed in holding tanks with running seawater at the Fish and Game Granite Canyon Lab. Control samples for both SO and TM are also held in the tank.

7.2.4 For freshwater clams: clams (25-50) are placed in 50mm X 7mm polypropylene mesh bags using identical procedures to those used with mussels (section 7.2.1). If clams need to be stored for more than 48 hours, the mesh bags are deployed in Lake San Antonio or another clean source until actual sample deployment.

7.2.5 The mussels are attached to an open water transplant system that consists of a buoy system constructed with a heavy weight anchor (about 100lbs) or screw-in earth anchor, 13mm polypropylene line, and a 30cm diameter subsurface buoy. The sample bags are attached with cable ties to the buoy line about 15 feet below the water surface. In some cases the sample is hung on suspended polypropylene lines about 15 feet below the water surface between pier pilings or other surface structures. Creosote-coated wooden piers are avoided because they are a potential source of contamination. In some cases the mussels are hung below a floating dock. In shallow waters a wooden or PVC stake is hammered into the substrate and the mussel bags are attached by cable ties to the stake.

7.2.6 The clams are deployed by attaching with cable ties the mesh bag to wooden or PVC stakes hammered into substrate or screw in earth anchors. The bags containing clams are typically deployed 15cm or more off the bottom. In areas of swift water, polypropylene line is also attached to the staked bags and a permanent object (piling, tree or rock).

7.2.7 Transplants are usually deployed for 1-4 months. Ideally mussels are transplanted in early September and retrieved in late December and early January. Clams are usually transplanted in March or April and retrieved in May or June.

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7.2.8 Data is recorded for each site samples are transplanted to or collected from. Data includes, but is not limited to station name, date collected or transplanted, collectors names, water depth, GPS readings, photo, ocean/atmospheric conditions (if appropriate), description of site, and drawing in necessary.

7.3 Sample Retrieval

7.3.1 The transplanted or resident and control mussels analyzed for metals are placed into two labeled Ziploc™ polyethylene bags (4mm thickness).

7.3.2 All mussels to be analyzed for organics are placed in an aluminum foil bag. The bags are constructed of two layers of "heavy duty" aluminum foil. Prior to use these bags are cleaned by heating to 500°c or by rinsing in petroleum ether or methanol. The sample is first wrapped in a foil bag, then placed in two labeled polyethylene Ziploc™ bags. Note: samples should only contact the dull side of the foil.

7.3.3 The bags containing samples are clearly and uniquely identified using a water-proof marking pen or pre-made label. Information items include ID number, station name, depth (if from a multiple sample buoy), program identification, date of collection, species and type of analysis to be performed.

7.3.4 The samples are placed in non-metallic ice chests and frozen using dry ice or regular ice. (Dry ice is used when the collecting trip takes more than two days.) At the lab, samples should be stored at or below -20°c until processed.

7.4 Sample Collection – Fish

7.4.1 Fish are collected using the appropriate gear for the desired species and existing water conditions.

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7.4.1.1 Electrofisher boat- The electrofisher boat is run by a trained operator, making sure that all on board follow appropriate safety rules. Once on site, adjustment of the voltage, amps, and pulse for the ambient water is made and recorded. The stainless steel fish well is rinsed with ambient water, drained and refilled. The shocked target fish are placed with a nylon net in the well with circulating ambient water. The nylon net is washed with a detergent and rinsed with ambient water prior to use. Between sites the net will be stored in a Ziploc™ bag. Electrofishing will continue until the appropriate number and size of fish are collected.

7.4.1.2 Backpack electrofisher- The backpack electrofisher is operated by a trained person, making sure that all others helping follow appropriate safety rules. The backpack electrofisher is used in freshwater areas where an electrofisher boat can not access. Once on site, adjustment of the voltage, amps, and pulse for the ambient water is made and recorded. The shocked target fish are captured with a nylon net and placed in a 30 gallon plastic bag. The nylon net is washed with a detergent and rinsed with ambient water prior to use. Electrofishing will continue until the appropriate number and size of fish are collected.

7.4.1.3 Fyke or hoop net- Six-36 inch diameter hoops connected with 1 inch square mesh net is used to collect fish, primarily catfish. The net is placed parallel to shore with the open hoop end facing downstream. The net is placed in areas of slow moving water. A partially opened can of cat food is placed in the upstream end of the net. Between 2-6 nets are placed at a site overnight. Upon retrieval a grappling hook is used to pull up the downstream anchor. The hoops and net are pulled together and placed on a 30 gallon plastic bag in the boat. With polyethylene gloves the desired fish are placed in a 30 gallon plastic bag and kept in an ice chest with ice until the appropriate number and size of fish are collected.

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7.4.1.4 Otter-trawl- A 14 foot otter trawl with 24 inch wooden doors or a 20 foot otter trawl with 30 inch doors and 80 feet of line is towed behind a boat for water depths less than 25 feet. For water depths greater than 25 feet another 80 feet of line was added to capture fish on or near the substrate. Fifteen minute tows at 2-3 knots speed are made. The beginning and ending times are noted on data sheets. The trawl is pulled over the side of the boat to avoid engine exhaust. The captured fish are emptied into a 30 gallon plastic bag for sorting. Desired fish are placed with polyethylene gloves into another 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.5 Gill nets- A 100 yard monofilament gill net of the appropriate mesh size for the desired fish is set out over the bow of the boat parallel to shore. The net is retrieved after being set for 1-4 hours. The boat engine is turned off and the net is pulled over the side or bow of the boat. The net is retrieved starting from the down-current end. If the current is too strong to pull in by hand, then the boat is slowly motored forward and the net is pulled over the bow. Before the net is brought into the boat, the fish are picked out of the net and placed in another 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.6 Beach seines- In areas of shallow water, beach seines of the appropriate length, height, and mesh size are used. One sampler in a wetsuit or waders pulls the beach seine out from shore. The weighted side of the seine must drag on the bottom while the float side is on the surface. The offshore sampler pulls the seine out as far as necessary and then pulls the seine parallel to shore and then back to shore, forming a half circle. Another sampler is holding the other end on shore while this is occurring. When the offshore sampler reaches shore the two samplers come together with the seine. The seine is pulled onto shore making sure the weighted side drags the bottom. When the seine is completely pulled onshore, the target fish are collected with polyethylene gloves and placed in a 30 gallon plastic bag and kept in an ice chest with ice. The beach seine is rinsed off in the ambient water and placed in the rinsed 30 gallon plastic bucket.

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7.4.1.7 Cast net- A 10 or 12 foot cast net is used to collect fish off a pier, boat, or shallow water. The cast net is rinsed in ambient water prior to use and stored in a covered plastic bucket. The target fish are sampled with polyethylene gloves and placed in a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.8 Hook and line- Fish are caught off a pier, boat, or shore by hook and line. Hooked fish are taken off with polyethylene gloves and placed in a Ziploc™ bag or a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.1.9 Spearfishing- Certain species of fish are captured more easily by SCUBA divers spearing the fish. Only appropriately trained divers following the dive safety program guidelines are used for this method of collection. Generally, fish in the kelp beds are more easily captured by spearing. The fish are shot in the head area to prevent the fillets from being damaged or contaminated. Spear tips are washed with a detergent and rinsed with ambient water prior to use.

7.4.1.10 Crab/lobster traps- Polyethylene traps are baited to collect crabs or lobsters. Traps are left for 1-2 hours. The crabs are placed in a Ziploc™ bag or a 30 gallon plastic bag and kept in an ice chest with ice.

7.4.2 As a general rule, five fish of medium size or three fish of larger size are collected as composites for analysis. The smallest fish length cannot be any smaller than 75% of the largest fish length. Five fish provides sufficient quantities of tissue for the dissection of 100 grams of fish flesh for organic and inorganic analysis. The medium size is more desirable to enable similar samples to be collected in succeeding collections.

7.4.3 When only small fish are available, sufficient numbers are collected to provide 100 grams of fish flesh for analysis. If the fish are too small to excise flesh, the whole fish, minus the head, tail, and guts are analyzed as composites.

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7.4.4 Fish collected, to large to fit in our clean bags (>500 mm) are initially dissected in the field. At the dock, the fish are laid out on a clean plastic bag and a large cross section from behind the pectoral fins to the gut is cut with a cleaned bone saw. The bone saw is cleaned (micro, DI, methanol) between fish and a new plastic bag is used. The internal organs are not cut into, to prevent contamination. For bat rays, a section of the wing is cut and saved. These sections are wrapped in Teflon ®, double bagged and packed in dry ice before transfer to the freezer. During lab dissection, a subsection of the cross section is removed, discarding any tissue exposed by field dissection.

7.4.5 Field data (Appendix A-2) recorded include, but are not limited to site name, sample identification number, site location (GPS), date of collection, time of collection, names of collectors, method of collection, type of sample, water depth, water and atmospheric conditions, fish total lengths (fork lengths where appropriate), photo number and a note of other fish caught.

7.4.6 The fish are then wrapped in cleaned Teflon sheets. The wrapped fish are then double-bagged in Ziploc™ bags with the inner bag labeled. The fish are put on dry ice and transported to the laboratory where they are kept frozen until they are processed for chemical analysis.

8.0 QUALITY CONTROL

8.1 Equipment Blanks: All equipment used in collection and preparation of samples is periodically checked for contamination. Before any new or different equipment is used it must be checked for contamination.

8.2 Sample Archive: All remaining sample homogenates and extracts are archived at -20°C for future analysis. A few of the more important original mussel and clam samples are also archived for future analysis.

8.3 Field Blanks: Field blanks are taken according to the specific study.

8.4 Field Replicates: Field replicates are taken according to the specific study. Generally, one field replicate is taken every twenty stations and is analyzed for both TM and SO.

8.5 A record of sample transport, receipt & storage is kept & available for reference.

8.6 All samples, once returned to the laboratory for processing, are prepared in a clean room to avoid airborne contamination.

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9.0 REFERENCES

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9.4 Hayes, S. P. and P. T. Phillips. 1986. California State Mussel Watch: Marine water quality monitoring program 1984-85. State Water Resources Control Board Water Quality Monitoring Report No. 86-3WQ.

9.5 EPA. 1995. Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories. Volume 1: Fish Sampling and Analysis. EPA 823-R-95-007.

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Appendix A-1 to SOP: Chain-of-custody form for tissue bioaccumulation analyses

Sampler	Phone #:	Contact Person:		Lab Number
	831-771-4162	Marco Sigala	Phone #: 831-771-4173	
Mailing Address		**Shipping Address**		**Field Number**
7544 Sandholdt Road		7544 Sandholdt Road	email: msigala@mlml.calstate.edu	
City **State** **Zip**		**City** **State** **Zip**		**Lab Storage**
Moss Landing CA 95039		Moss Landing CA 95039		
		Mailing Address		**Spill Title**
Date Required/Reason		**City** **State** **Zip**		**Suspect**
		Moss Landing CA 95039		
Shipped Via				**Index-PCA**

☐ **SWAMP Region:**_____ **Fiscal Year:** _____ **Leg:** _____

☐ **Project ID:** _____

☐ **Season:** _____ **Event:** _____

Water Temp: **F or C** **pH:** **DO:** **mg/L Conductivity:** ***u*** **mhos/cm**

Analysis Requested

Sample Identification/Location (Draw map on separate sheet if necessary)	Collection Date	Collection Time	Bioaccumulation, mussels	Bioaccumulation, fish	Bioaccumulation, corbicula (specify)	Bioaccumulation, other n(specify)		Please provide separate list of species collected and attach to COC.				Sample Type: Water	Sample Type: Filtered Water	Sample Type: Soil	Sample Type: Filter	Number of Containers: Plastic	Number of Containers: Glass	Number of Containers: VOA Vial	Number of Containers: Foil Wrap	Preservation: Temp	Preservation: Acid	

Problem Description

Suspect/Incident Location

Comments/Special Instructions

Pollution Action Kit: Yes☐ No☐

Glove Size: Large ☐ Medium ☐

Hazmat Shipper Requested: Yes☐ No☐

Samples Relinquished By (Signature)	Print Name	Date	Received By (Signature)	Print Name

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Appendix A-2 to SOP: Field Data Sheet for tissue bioaccumulation samples

SWAMP Bioaccumulation

PG: OF PGS

Entered in Dbase

*Station ID:

*Sample Code:

*Date: Collection for Bioaccumulation M M D D Y Y Y Y

*Sample Time:

*Species
Corbicula
M. Californicus
other _____

*Resident / Transplant

Deployment

*Deployment Date: M M D D Y Y Y Y

Crew:

Deployment Time:

Transplant origin:

*Equipment Type
PVC
Rebar
screwin earth anchor
polypro

Water Depth (m) :

Stream Width (m) :

Sample depth below surface (m):

Number transplanted:

Precipitation
dry
drizzle
rain
thunderstorm

Sky
clear
partly cloudy
overcast
fog

Water Clarity
clear
semi-clear
turbid

Water Movement
still
fast
slow

Habitat
dry
non-wadeable stream
wadeable stream
wadeable concrete channel
standing water
other_____

Retrieval/Collection

*Retrieved/ Non-Retrieved

Estimated Number Survival:

*Sample depth from surface (m):

*Sample depth from bottom (m):

Sample used for

*organics Yes / No

*metals Yes / No

Comments: (if samples not retrieved; record as sample failure)

*** required field; underlined fields used as primary keys in dbase SWAMP SFDS v2 2/6/2002**

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Appendix A-3 to SOP: Fish measurement form for laboratory processing

Lab #: _____ **Stn #:** _____

Stn Name: _____

Date Coll: _____ **Species:** _____

Date Diss: _____ **Skin ON/OFF:** _____

Initials: _____ **Comp/Ind:** _____

Fish#	Weight (g)	Total Length (mm)	Fork Length (mm)	Fillet Weight (g)	Sex	Comments
1					M / F	
2					M / F	
3					M / F	
4					M / F	
5					M / F	
6					M / F	
7					M / F	
8					M / F	
9					M / F	
10					M / F	

Jar Wt (Empty): _____

Jar Wt (Full): _____

Sample Wt: _____